

# The effects of particle size, fermentation and roasting of cocoa nibs on supercritical fluid extraction of cocoa butter

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## Abstract

The effects of particle size, fermentation, roasting time and roasting temperature of cocoa nibs on cocoa butter extraction using supercritical fluid technology were studied. The effect of particle size was studied using cocoa liquor ( $D = 0.074$  mm), ground cocoa nib ( $D = 0.25$ – $0.50$  mm and  $1.0$ – $1.2$  mm) and whole cocoa nibs at 35 MPa, 60 °C and flow rate of 2 ml/min using supercritical carbon dioxide (SC-CO<sub>2</sub>). The effect of degree of fermentation was studied using unfermented, partly fermented and fermented cocoa, whereas the effect of roasting using roasted and unroasted cocoa nibs. Fermentation and roasting studies were conducted under the same operation conditions as particle size study using SC-CO<sub>2</sub> but with ethanol (25% w/w) as cosolvent. Cocoa butter extracted from the three studies was analyzed for total fat content (%), triglycerides and fatty acid methyl ester. The results showed that the extraction yield was significantly increased by a reduction in particle size. The highest yield was also obtained using unfermented cocoa, roasted for 35 min and at 150 °C. Generally, cocoa butter had similar triglycerides and fatty acid methyl ester composition at 5, 10 and 15 h extraction time. Glycerol-1,3-dipalmitate-2-oleate (POP), glycerol-1-palmitate-2-oleate-3-stearate (POS), and glycerol-1,3-distearate-2-oleate (SOS) account for most of the triglycerides, with POS (42.52–46.44%) being the major component. Palmitic, stearic and oleic were the main fatty acids in the extracted cocoa butter, with stearic acid being the highest component (33.70–40.22%).

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**Keywords:** Cocoa butter; Particle size; Fermentation; Roasting; Supercritical fluid extraction

## 1. Introduction

Cocoa beans consist mainly of cocoa butter (50–55% w/w); high quality cocoa butter used in food, cosmetic and pharmaceutical products is obtained by mechanical press, expeller, and solvent extraction using hexane. Increasing awareness of health and safety hazard associated with the use of organic solvents, due to its possible contamination of extracted products has placed a new demand on the food industry to develop new and clean technologies for processing of food products (Atta & Choudhary, 1990; Belitz & Grosch, 1987; Rossi, 1996; Saldana, Mohamed, & Mazzafera, 2002). Supercritical fluid

extraction (SFE) using carbon dioxide as a solvent has provided an excellent alternative to the use of chemical solvent in the extraction of cocoa butter from different plant matrices. In comparison with established methods, SFE has some important advantages, particularly in its ability to yield products that are completely free from processing residues. SFE is also an alternative from the standpoint of time-saving and for environmental reasons such as the reduction of the use of large solvent volumes. Carbon dioxide (CO<sub>2</sub>) is an ideal solvent for extraction of natural products because it is nontoxic, non-explosive, readily available, and easily removable from the products (Simandi, Deak, & Ronyai, 1999).

Previous studies have indicated that processing have caused differences in physico-chemical compositions of cocoa beans and the characterization of cocoa matrix

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(Amin, Jinap, & Jamilah, 1997, 1998; Amin, Jinap, Jamilah, Harikrisna, & Biehl, 2002; Biehl & Voigt, 1996; Hoskin & Dimick, 1984, 1994; Jinap, 1994; Puziah, Jinap, Sharifah, & Asbi, 1998; Rohan, 1963). Hence, the nature of the sample matrix can have a profound effect on the results obtained with SFE. Unfortunately, knowledge of analyte solubility in supercritical fluids does not always allow a prediction to be made as to the effectiveness of SFE for extracting a particular matrix (Wenclawiak, 1992). In cocoa bean processing, many treatments are used to produce matrix characteristics of cocoa products, such as fermentation, drying, roasting, winnowing, milling/grinding and alkalization. Fermentation is a very important aspect of cocoa processing for the production of flavor precursors, whereas roasting is very important in the development of flavor and aroma (Jinap, Dimick, & Hollender, 1995). Bean from some origins is only partially fermented or may even be completely unfermented. Generally these are of little interest to the chocolate manufacturer (Hancock, 1994). Winnowing is a critical step that is performed to separate the valuable product (e.g., nibs) from the by-product (i.e., shell). Milling converts nibs into a fluid paste, known as liquor, which is hydraulically pressed for cocoa butter production. Alkalization is used mainly to change the color and the beans, liquor, nibs or powder are often treated with solutions or suspensions of alkali, usually potassium or sodium carbonate (Dimick & Hoskin, 1981).

Rossi (1996), McHugh and Krukoni (1994) reported that cocoa butter can be extracted from liquor and shells using supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) at 30–40 MPa and 50–80 °C; they also found that the extraction yield was depended on the degree of disruption of lipid-bearing cells. They observed that the fatty acid and triglyceride composition of cocoa butter extracted were within the range required for this product and that the pyrazine fraction was almost equally distributed between the solid and the supercritical phase during extraction. However, the SC-CO<sub>2</sub> extraction did not reduce the aroma of the residue with respect to the pyrazine fraction (McHugh & Krukoni, 1994; Roselius, Vitzthum, & Hubert, 1975). So far, however, there has been little discussion about study sample matrix on cocoa butter extraction. Types of sample matrix of processed cocoa beans would be used as variable in the study of cocoa butter extraction using supercritical fluid technology.

Most of the recent studies on cocoa butter extraction using SFE have been carried out in the pressure range of 15–40 MPa with temperature of 40–60 °C (Li & Hartland, 1996; Roselius et al., 1975; Rossi, 1996). The results indicated that the higher amount of extracted cocoa butter was obtained when the higher pressure was used at the temperature range being studied. The study also indicated that the addition of cosolvent to SC-CO<sub>2</sub> such as ethanol, a well known polar cosolvent for its application in food and natural product improved the efficiency of the cocoa butter extraction. Li and Hartland (1992) reported that the addition of 20–25% w/w ethanol as cosolvent has greatly enhanced solubilities of cocoa butter and its extraction

from cocoa matrix to a maximum yield; however, the yield remained almost constant at 25–33% w/w ethanol. In this study, SC-CO<sub>2</sub> was performed at 35 MPa and 60 °C using 25% w/w ethanol as cosolvent. Therefore, the aim of this study was to determine the effect of particle size, degree of fermentation and roasting of cocoa nibs on the efficiency of cocoa butter extraction using SC-CO<sub>2</sub> along with ethanol as cosolvent.

## 2. Materials and methods

### 2.1. Sample preparation

Cocoa bean samples were purchased from K.L. Kepong Sdn. Bhd., Port Klang Malaysia. Liquid CO<sub>2</sub> was obtained from Malaysian Oxygen (MOX), Petaling Jaya, Selangor, Malaysia. Chromatography grade solvent was used in high pressure liquid chromatography (HPLC) and gas chromatography (GC) analysis. Standard of triglycerides, fatty acid methyl esters, ethanol, ethylene glycol, petroleum ether, methanol, acetone and acetonitrile were purchased from Sigma, Merck, and Fisher (Malaysia). In the study on particle size, fermented dried beans were deshelled and ground in Waring blender (Braun, Germany) in the presence of liquid nitrogen, then passed through a mesh sieve (diameter ~0.074 mm; 0.25–0.50 mm and 1.00–1.20 mm) as quickly as possible. Whole nibs were obtained by deshelling of beans manually. Fermentation study was carried out on unfermented, partly fermented (2 days fermentation) and fully fermented (6 days fermentation) dried beans that were deshelled and ground in a Waring blender (Braun, Germany) in the presence of liquid nitrogen before being passed through a mesh sieve (1.00–1.20 mm) as quickly as possible. Ground fermented cocoa nibs with 1.00–1.20 mm diameter particle size were used for the study on the effect of roasting. Roasting of ground cocoa nibs was carried out in an oven (Memmert UL 40, Germany), which was set and maintained at specified temperature and time of roasting (120 °C, 15 min; 120 °C, 25 min; 120 °C, 35 min; 150 °C, 15 min; 150 °C, 25 min and 150 °C, 35 min). Fifty grams of cocoa nibs were placed in a 10 cm glass petri dish at 5 mm thickness before being placed in the oven. After roasting, samples were placed in a desiccators containing silica gel at ambient temperature (26 °C) for further experiment.

### 2.2. SFE procedure for cocoa butter extraction

The SFE apparatus consisted of Intelligent HPLC Pump Model PU-1580 (Jasco Corporation, Tokyo, Japan) fitted with a cooling jacket to deliver CO<sub>2</sub>. In order to cool the pump head, ethylene glycol–deionized water mixture (50:50, v/v) was circulated through the cooling jacket using a Low Temperature Bath Circulator Model 631D (Tech-Lab Manufacturing Sdn. Bhd., Selangor, Malaysia) which can deliver coolant down to –20 °C. A 10 g sample was loaded into a 50 ml Extraction Vessel Model EV-3 (Jasco

Corporation, Tokyo, Japan) that was placed in Column Oven Model CO-1560 (Jasco Corporation, Tokyo, Japan). The column oven was used to maintain the extraction temperature. A Back Pressure Regulator (BPR) Model BP-1580-81 (Jasco Corporation, Tokyo, Japan) was used to control the extraction pressure. The cocoa butter dissolved in the supercritical fluid was separated from the carbon dioxide while it was in the separator before being collected in a 100 ml Schott bottle. The collection of cocoa butter occurred during depressurizing of the separator using smooth/slow spray mode; the exhausting of CO<sub>2</sub> was held at 60 °C. Separation was controlled using Back Pressure Regulator (BPR) Model BP-1580-81 (Jasco Corporation, Tokyo, Japan). Cocoa butter was collected every 1 h. In the study on the effect of particle size, extraction of cocoa butter from the sample was performed by SFE using SC-CO<sub>2</sub> at 60 °C, 35 MPa pressure and flow rate of 2 ml/min. In fermentation and roasting studies, extraction of cocoa butter from the sample was performed using ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 60 °C, 35 MPa pressure and flow rate of 2 ml/min.

### 2.3. Determination of yield

The initial weight of the Schott bottle used to collect cocoa butter samples from SFE was measured gravimetrically. After SFE extraction, the Schott bottle containing the extracted fat was transferred into a desiccator at room temperature until the constant final weight was obtained. The residue of the cosolvent of the extracted fat was evaporated under vacuum at temperature of 70 °C using a Heidolph WB/VV 2000 Rotary Evaporator (Heidolph, Germany); the extracted fat was later placed in the oven at 45 °C for 30 min before being transferred into the desiccators. Yield was calculated by dividing the percentage of cocoa butter in the samples with 53% factor (amount of cocoa butter in cocoa nibs, determined by Soxhlet method, AOAC, 1998).

$$\text{Yield} = \frac{\left[ \frac{\text{weight of fat}}{\text{weight of sample}} \right] \times 100\%}{53\%}$$

### 2.4. Determination of triglycerides

Triglycerides composition was determined by HPLC according the AOCS method (AOCS, 1993). Ten percent cocoa butter solution was prepared using acetone as solvent. The solution was then filtered using TE 36 membrane filter (PTFE; 0.45 µm) (Millipore) before being filtered through the Sep-Pak Plus Silica cartridge (Waters) to discard any impurities. Determination of triglyceride was conducted using Waters HPLC instrument, Waters Associates Model 600 Controller and Model 410 RI Detector (Waters, USA), Waters HPLC C18 column (3.9 mm id × 300 mm length), column temperature of 30–35 °C, column pressure of 5–6 MPa, mobile phase (acetone/acetonitrile 75:25 v/v),

flow rate of mobile phase of 1 ml/min and injection volume of 10 µl. The value of triglycerides was expressed as a percentage. Analysis was carried out at 5, 10 and 15 h extraction time of cocoa butter with three replications.

### 2.5. Determination of fatty acid methyl esters (FAMES)

The fatty acids composition of fat mixtures was determined as fatty acid methyl esters (FAMES) using gas chromatography and Restek Rtx-2330 column (30 m; 0.25 mm; 0.2 µm). FAMES were prepared by dissolving 0.05 g sample into 0.8 ml petroleum ether (b.p. 40–60 °C, Merck Malaysia) and 0.2 ml of 1 M sodium methoxide (30% methanol in sodium methoxide) was added. The mixture was then shaken gently using an autovortex (Stuart, UK) for 30 s and stored for 5 min for it to form two layers. A volume of 0.5 µl of the upper layer was injected into an injector port of gas chromatography GC HP 5890 A (Hewlett Packard, Wilmington, USA) with the temperature program that started at 115 °C, a heating rate of 6 °C/min and a final temperature of 200 °C (AOCS, 1993). Analysis was carried out at 5, 10 and 15 h extraction time of cocoa butter with three replications.

### 2.6. Statistical analysis

Statistical analysis was carried out using the Statistical Analysis System (SAS, 1999) Version 8 (TS M1) by SAS Institute Inc., Cary, NC, USA for analysis of variance (ANOVA) and significant differences between means for all treatments (Duncan's multiple range test) at a level of  $P < 0.05$ .

## 3. Results and discussion

The rate of removal of solute from a sample matrix using SFE is a function of its solubility in the fluid media and the rate of mass transport of the solute out of the sample matrix. Study on the geometric size of matrix particles can influence the speed and completeness with which SFE can be conducted. The effect of particle size on cocoa butter extraction using SC-CO<sub>2</sub> is shown in Fig. 1. The results showed that the yield of cocoa butter extracted from the smaller particle sized cocoa nibs (S1 = 0.074 mm, S2 = 0.25–0.50 mm and S3 = 1.00–1.20 mm) were significantly ( $P < 0.05$ ) higher compared to that of the whole cocoa nib (S4), with S1 having the highest yield. The initial rate of extraction of S1 and S2 increased rapidly for almost 10 h with 70% and 58% of yield, respectively; after that, the rate increased slowly for almost 20 h with 92% and 78% of yield, respectively, and finally for more than 20 h the yield for S1 and S2 remained constant. These results demonstrated that extraction of yield increased with the reduction in particle size of cocoa sample during the first 20 h of extraction time. However, the extraction time was too long if it is to be performed in the real application; hence the addition of cosolvent to speed up the extraction is necessary.

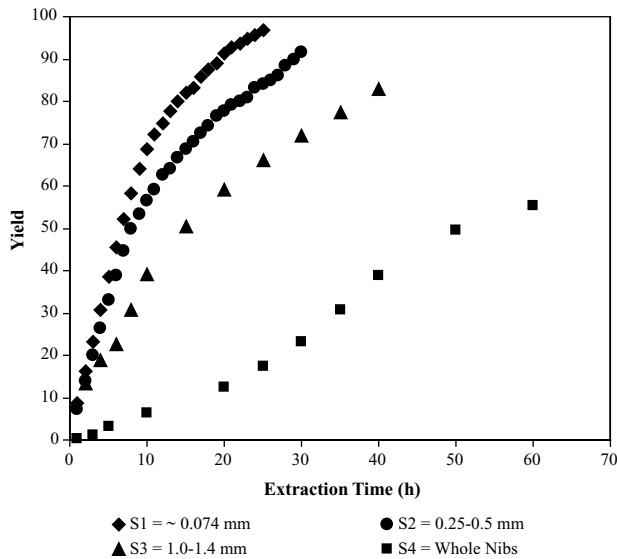


Fig. 1. The yield of cocoa butter extracted using SC-CO<sub>2</sub> at pressure ( $P$ ) = 35 MPa, temperature ( $T$ ) = 60 °C and flow rate ( $f$ ) = 2 ml/min as a function of the extraction time with different particle size.

Cocoa liquor (S1) which has a small physical morphology of the matrix would have a better influence on the efficiency of extraction and the rate at which it was conducted. This effect normally results from shorter internal diffusion path lengths over which the extracted solutes must travel to reach the fluid phase (Taylor, 1996). This view is supported by Bravi et al. (2007) who reported an increase in  $\alpha$ -tocopherol-enriched oil yield from grape seeds by supercritical CO<sub>2</sub>; this effect can be explained with the increase in the available surface area for mass transfer and with the reduction of the time required for the initial soaking of the vegetal matrix. The increase in the oil yield was also attributed to the decreased diffusional resistance in the solid phase of smaller seed fragments. Furthermore, Bernardo-Gil et al. (2007) reported that the rate of corn oil extraction by SC-CO<sub>2</sub> depends on the particle size of the corn matrix. Smaller particles would have greater fluid–solid contact areas and hence greater rate of oil extraction.

The extraction efficiency depends on transfer of analytes from their host matrices to the supercritical fluid and flushing of analytes from the extraction cell by the supercritical fluid. The mass transfer of analytes flushed out of the sample matrix depends on the mass transfer resistance (Lees & Jackson, 1975; Wenclawiac, 1992). Solubility of analytes becomes important when analyte–matrix interaction occur during extraction. The findings for the SC-CO<sub>2</sub> extraction of cocoa liquor in the study are consistent with those reported by Li and Hartland (1996), who measured cocoa butter solubility in SC-CO<sub>2</sub> directly on the product prepared by hydraulic expressing of cocoa. The study confirmed the involvement of leaching mechanism during the SC-CO<sub>2</sub> extraction of cocoa butter from such a fine-milled product (cocoa liquor). Once fat is released from the lipid-bearing cells (through the fine-grinding process), SFE can be readily applied for the extraction of the fat (Rossi, 1996).

Fig. 2 shows the effect of fermentation on cocoa butter extraction using ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub>. The results indicated that yield of cocoa butter extracted from unfermented ground cocoa nib (F1) was the highest with significant value ( $P < 0.05$ ) compared to those of partly and fully fermented (F2 and F3, respectively) samples. Sample F1 achieved 100% yield after extraction for nearly 10 h. Fermentation is the essential first step in development of cocoa flavor precursors. During cocoa fermentation, the bean death caused cellular membranes break down, allowing the different constituents and enzymes, which were kept separate in the living tissue, to come into contact and interact to produce flavor precursors; these substances would affect the extraction by supercritical fluid. Unfermented cocoa beans did not produce any characteristic cocoa and chocolate flavor during roasting. Bicking, Hayes, Kiley, and Deming (1993) and

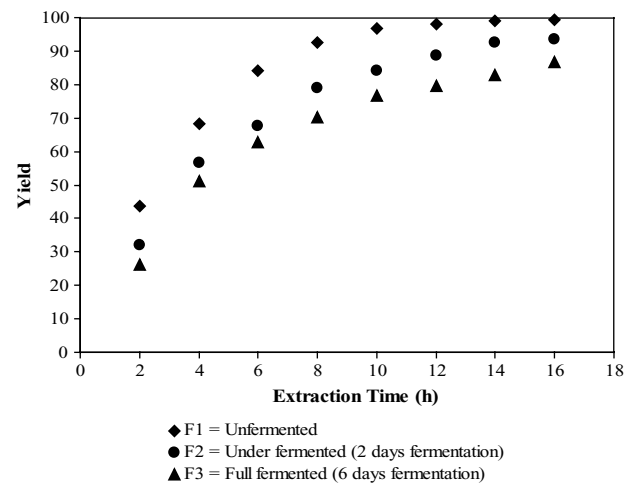


Fig. 2. The yield of cocoa butter extracted using ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 ml/min as a function of the extraction time with different degree of fermentation.

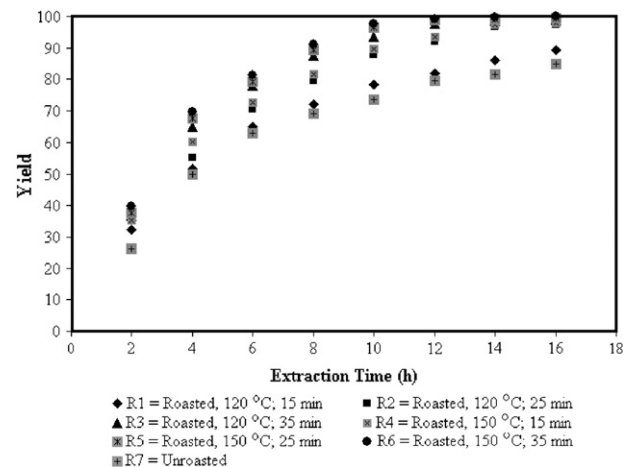


Fig. 3. The yield of cocoa butter extracted using ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 ml/min as a function of the extraction time with different roasting treatment.

Brunner (1994) reported that solubility of low volatile substance in unpolar supercritical gasses decreased with increasing molecular mass, polarity and number of polar functional groups. At higher molecular weight (MW), the influence of the unpolar part of the molecules which is proportional to MW, dominates solubility while at lower MW, the influence of functional groups dominates solubility. Jinap (1994) reported that the volatile acids (acetic, propionic,

butyric, isobutyric and isovaleric) and nonvolatile acids (citric, lactic, malic, succinic, oxalic and tartaric) were produced in the pulp through sugar degradation by the metabolism of microorganisms absorbed into the cotyledon during fermentation. In addition, during fermentation the degradation of sugar, polysaccharides and protein by proteolytic enzymatic would result in the formation of monosaccharides (fructose and glucose) and amino acids

Table 1  
Triglyceride composition (area %)<sup>1</sup> of cocoa butter extracted by supercritical carbon dioxide (SC-CO<sub>2</sub>) at 35 MPa, 60 °C and 2 ml/min of flow rate with different particle size and extraction time

| Sample <sup>2</sup> | 5 h Extraction time |         |         |                     | 10 h Extraction time |          |         |                     | 15 h Extraction time |         |         |                     |
|---------------------|---------------------|---------|---------|---------------------|----------------------|----------|---------|---------------------|----------------------|---------|---------|---------------------|
|                     | POP                 | POS     | SOS     | Others <sup>3</sup> | POP                  | POS      | SOS     | Others <sup>3</sup> | POP                  | POS     | SOS     | Others <sup>3</sup> |
| S1                  | 21.38Ab             | 44.19Aa | 28.69Ab | 5.73Ba              | 21.53Aa              | 43.39Bc  | 28.38Ac | 6.69Aa              | 21.42Aa              | 43.16Bb | 28.74Ab | 6.68Aa              |
| S2                  | 21.60Aab            | 43.51Bb | 30.17Aa | 4.73Bd              | 20.27Bb              | 45.17Aa  | 30.16Aa | 4.40Ad              | 20.41Bb              | 44.85Aa | 29.57Ba | 5.17Ab              |
| S3                  | 21.74Aa             | 43.31Bb | 30.02Aa | 4.93Ac              | 21.27Ba              | 44.69Aab | 29.48Bb | 4.57Bc              | 21.12Ba              | 45.12Aa | 28.78Cb | 4.98Ac              |
| S4                  | 21.69Aa             | 43.22Cb | 29.96Aa | 5.12Ab              | 21.42ABa             | 44.53Bb  | 29.15Bb | 4.89Bb              | 21.29Ba              | 45.42Aa | 28.42Cb | 4.87Bd              |

<sup>A-C</sup> Means within a row with different letters are significantly different ( $P < 0.05$ );

<sup>a-d</sup> Means within a column with different letters are significantly different ( $P < 0.05$ ).

<sup>1</sup> Means of three replications.

<sup>2</sup> Cocoa butter extracted obtained by SC-CO<sub>2</sub> with <0.070 mm particle size (S1) of cocoa liquor, 0.25–0.50 mm particle size (S2) of ground cocoa nibs, 1.00–1.40 mm particle size (S3) of ground cocoa nibs and whole nibs (S4).

<sup>3</sup> PLiO, PLiP, POO, SOO, and SOA where P = palmitic, O = oleic, S = stearic, Li = linoleic.

Table 2  
Triglyceride composition (area %)<sup>1</sup> of cocoa butter extracted by ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> 35 MPa, 60 °C and 2 ml/min of flow rate with different fermentation treatment and extraction time

| Sample <sup>2</sup> | 5 h Extraction time |         |         |                     | 10 h Extraction time |         |         |                     | 15 h Extraction time |         |         |                     |
|---------------------|---------------------|---------|---------|---------------------|----------------------|---------|---------|---------------------|----------------------|---------|---------|---------------------|
|                     | POP                 | POS     | SOS     | Others <sup>3</sup> | POP                  | POS     | SOS     | Others <sup>3</sup> | POP                  | POS     | SOS     | Others <sup>3</sup> |
| F1                  | 19.70Aa             | 43.88Aa | 29.73Bb | 6.69Aa              | 19.00Ba              | 43.97Ab | 31.50Aa | 5.52Ba              | 19.88Aab             | 44.11Aa | 31.42Aa | 4.59Ca              |
| F2                  | 21.79Aa             | 43.41Aa | 30.09Aa | 4.71Aa              | 20.58Aa              | 45.06Aa | 29.73Aa | 4.63Aa              | 19.20Ab              | 46.44Aa | 29.25Aa | 5.11Aa              |
| F3                  | 21.74Aa             | 43.31Aa | 30.03Aa | 4.92Aa              | 21.43Aa              | 44.58Aa | 29.41Aa | 4.58Aa              | 21.08Aa              | 45.36Aa | 28.57Aa | 4.99Aa              |

<sup>A-C</sup> Means within a row with different letters are significantly different ( $P < 0.05$ );

<sup>a-c</sup> Means within a column with different letters are significantly different ( $P < 0.05$ ).

<sup>1</sup> Means of three replications.

<sup>2</sup> Cocoa butter extracted obtained by ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 ml/min of flow rate with unfermented cocoa (F1), partly fermented (F2) and full fermented (F3).

<sup>3</sup> PLiO, PLiP, POO, SOO, and SOA.

Table 3  
Triglyceride composition (area %)<sup>1</sup> of cocoa butter extracted by ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 ml/min of flow rate with different roasting treatment and extraction time

| Sample <sup>2</sup> | 5 h Extraction time |          |          |                     | 10 h Extraction time |          |          |                     | 15 h Extraction time |          |         |                     |
|---------------------|---------------------|----------|----------|---------------------|----------------------|----------|----------|---------------------|----------------------|----------|---------|---------------------|
|                     | POP                 | POS      | SOS      | Others <sup>3</sup> | POP                  | POS      | SOS      | Others <sup>3</sup> | POP                  | POS      | SOS     | Others <sup>3</sup> |
| R1                  | 21.90Ab             | 44.01Ab  | 28.64Cd  | 5.46Bc              | 21.40Ba              | 43.64Acd | 29.26Bde | 5.70Ac              | 20.33Cc              | 44.11Ab  | 30.28Ab | 5.28Cc              |
| R2                  | 21.85Ab             | 44.26Aab | 29.14Bc  | 4.75Be              | 21.72Aa              | 43.13Bde | 28.80Be  | 6.35Ab              | 20.22Bc              | 44.83Aa  | 30.47Ab | 4.48Ce              |
| R3                  | 22.59Aa             | 42.94Bc  | 27.78Be  | 6.68Aa              | 21.49Bc              | 43.88Abc | 29.91Ac  | 4.72Ce              | 20.74Cb              | 44.12Ab  | 30.08Ab | 5.07Bd              |
| R4                  | 20.90Ac             | 42.52Cc  | 29.90Ab  | 6.67Aa              | 20.18Bc              | 44.87Aa  | 29.91Ac  | 5.04Cd              | 21.18Aa              | 43.34Bcd | 30.17Ab | 5.32Bc              |
| R5                  | 19.44Ad             | 44.24Aab | 30.75Ca  | 5.58Ab              | 19.15ABd             | 43.99Abc | 32.10Ba  | 4.77Ce              | 18.91Be              | 42.98Bd  | 33.05Aa | 5.06Bd              |
| R6                  | 19.72Ad             | 44.82Aa  | 30.18Ab  | 5.28Bd              | 19.87Ac              | 42.62Be  | 30.57Ab  | 6.94Aa              | 19.87Ad              | 42.62Bd  | 30.57Ab | 6.94Aa              |
| R7                  | 20.70Bc             | 43.65Bb  | 29.05Acd | 6.60Aa              | 20.89ABb             | 44.58Aab | 29.41Ad  | 5.12Cd              | 21.14Aa              | 43.78Bbc | 29.46Ac | 5.62Bb              |

<sup>A-C</sup> Means within a row with different letters are significantly different ( $P < 0.05$ );

<sup>a-e</sup> Means within a column with different letters are significantly different ( $P < 0.05$ ).

<sup>1</sup> Means of three replications.

<sup>2</sup> Cocoa butter extracted obtained by ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 ml/min of flow rate with roasted sample of 120 °C; 15 min (R1), 120 °C; 25 min (R2), 120 °C; 35 min (R3), 150 °C; 15 min (R4), 150 °C; 25 min (R5), 150 °C; 35 min (R6) and unroasted treatment (R7).

<sup>3</sup> PLiO, PLiP, POO, SOO, and SOA.

(leucine, alanine, phenylalanine and tyrosine) (Amin et al., 1997; Amin, Jinap, & Jamilah, 1998; Amin et al., 2002). These precursors and products would affect the solubility of fat during SC-CO<sub>2</sub> extraction (Brunner, 2005 & Moyler, 1993). It has been demonstrated that full fermented and partly fermented treatment had lower extraction rate as compared to the unfermented treatment.

Fermentation and drying are two primary steps of cocoa bean processing, which are very important in producing aroma precursors and reducing astringency and bitterness. The production of aroma precursors is a vital stage to produce full aroma of cocoa products and cocoa aroma is only developed during the roasting of fermented cocoa beans (Biehl & Voigt, 1996; Hoskin & Dimick, 1984, 1994; Jinap, Wan Rosly, Russly, & Nurdin, 1998; Puziah et al., 1998; Rohan, 1963). Cocoa aroma was successfully generated *in vitro* from its precursors in the presence of cocoa butter, although the real chocolate flavor was not obtained (Biehl & Passern, 1982). During roasting of cocoa beans, the pressure from escaping internal gases, including water vapor, disrupts cellular structures, making the seed more brittle and causes loss of adhesive quality of the hull. During the roasting process, some chemical reactions take place resulting in the characteristic chocolate flavor of the final product. Since many of these compounds are water-miscible, they are evaporated off with water causing the moisture content of sample matrix to decrease (King & France, 1992; Simandi et al., 1999). This condition made it easy for solvent diffusion through the cell internal of the cocoa matrix for the cocoa butter to be extracted in shorter period of time. Therefore, roasting treatment of cocoa is an important step in enhancing the extraction of cocoa butter using supercritical fluid.

The yield of cocoa butter extracted from the roasted and unroasted ground cocoa nibs using ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> are shown in Fig. 3. The study found that roasted ground cocoa nibs extracted using SC-CO<sub>2</sub> (R1–R6) produced significantly higher ( $P < 0.05$ ) yield of cocoa butter compared to the unroasted sample (R7); sample R6 (roasting of 150 °C and 35 min) achieved 100% yield after extraction for nearly 14 h. This indicates that increasing roasting time and temperature had resulted in an increase in yield of the extracted cocoa butter. This may be due to the increased solubility of cocoa butter in SC-CO<sub>2</sub> solvent at lower moisture content. The effect of moisture on the extraction of analytes from biological tissues during SFE has been a point of controversy for some time. However, it appears that partial dehydration of the sample matrix will allow a more rapid SFE. This is due to the fact that highly hydrophilic matrices inhibit contact between the supercritical fluid and the target analytes. King and France (1992) have demonstrated that the removal of water can have a dramatic effect on the recovery of lipid moieties from meat product. On the other hand, higher temperature and longer roasting time would result in the increase of matrix porosity and weaker cocoa butter–matrix interaction. For this reason, an increase in the matrix porosity will

Table 4  
Fatty acids composition (area %)<sup>1</sup> of cocoa butter extracted by supercritical carbon dioxide (SC-CO<sub>2</sub>) at 35 MPa, 60 °C and 2 ml/min of flow rate with different particle size and extraction time

| Sample <sup>2</sup> | 5 h Extraction time |         |          |        |                     | 10 h Extraction time |         |         |        |                     | 15 h Extraction time |         |         |        |                     |
|---------------------|---------------------|---------|----------|--------|---------------------|----------------------|---------|---------|--------|---------------------|----------------------|---------|---------|--------|---------------------|
|                     | C16:0               | C18:0   | C18:1    | C18:2  | Others <sup>3</sup> | C16:0                | C18:0   | C18:1   | C18:2  | Others <sup>3</sup> | C16:0                | C18:0   | C18:1   | C18:2  | Others <sup>3</sup> |
| S1                  | 29.61Bc             | 39.35Aa | 27.35Ac  | 2.13Cc | 1.57Ab              | 30.38Ab              | 39.53Ba | 26.36Bc | 2.29Ab | 1.44Bc              | 29.82Bb              | 40.22Ba | 26.40Bc | 2.23Bb | 1.32Cc              |
| S2                  | 30.36Bb             | 38.12Ab | 28.04Ab  | 2.18Cb | 1.46Ac              | 30.58Ab              | 39.18Ba | 26.53Bc | 2.30Ab | 1.41Bd              | 30.00Bb              | 39.85Ba | 26.57Bc | 2.25Bb | 1.33Cc              |
| S3                  | 30.83Ab             | 36.66Bc | 29.24Ba  | 2.03Ad | 1.23Cd              | 29.62Bc              | 36.73Ab | 30.31Aa | 1.85Bc | 1.50Bb              | 28.46Cc              | 39.90Ca | 28.41Ca | 1.70Cc | 1.53Ab              |
| S4                  | 32.95Aa             | 34.19Ad | 27.63Abc | 2.47Aa | 2.77Ba              | 32.73Aa              | 33.94Ac | 28.07Ab | 2.48Aa | 2.77Ba              | 33.14Aa              | 33.70Ab | 27.66Ab | 2.36Ba | 3.14Aa              |

A–C Means within a row with different letters are significantly different ( $P < 0.05$ );  
a–d Means within a column with different letters are significantly different ( $P < 0.05$ ).  
<sup>1</sup> Means of three replications.  
<sup>2</sup> Cocoa butter extracted obtained by SC-CO<sub>2</sub> with <0.070 mm particle size (S1) of cocoa liquor, 0.25–0.50 mm particle size (S2) of ground cocoa nibs, 1.00–1.40 mm particle size (S3) of ground cocoa nibs and whole nibs (S4).  
<sup>3</sup> C12:0, C14:0 and C18:3.

Table 5  
Fatty acids composition (area %)<sup>1</sup> of cocoa butter extracted by ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 mL min<sup>-1</sup> of flow rate with different fermentation treatment and extraction time

| Sample <sup>2</sup> | 5 h Extraction time |          |         |        |                     | 10 h Extraction time |          |          |        |                     | 15 h Extraction time |         |         |        |                     |
|---------------------|---------------------|----------|---------|--------|---------------------|----------------------|----------|----------|--------|---------------------|----------------------|---------|---------|--------|---------------------|
|                     | C16:0               | C18:0    | C18:1   | C18:2  | Others <sup>3</sup> | C16:0                | C18:0    | C18:1    | C18:2  | Others <sup>3</sup> | C16:0                | C18:0   | C18:1   | C18:2  | Others <sup>3</sup> |
| F1                  | 29.31Aa             | 36.36Bb  | 30.63Aa | 2.18Aa | 1.52Ca              | 28.24Bc              | 37.67Aa  | 30.11Aa  | 2.22Ab | 1.76Ba              | 28.47Bc              | 37.38Aa | 30.10Aa | 2.20Aa | 1.86Aa              |
| F2                  | 28.53Cb             | 37.39Aa  | 30.48Aa | 2.14Bb | 1.46Cb              | 29.12Bb              | 37.02Aab | 29.96ABa | 2.31Aa | 1.60Ab              | 29.77Aa              | 36.78Aa | 29.79Ba | 2.13Bb | 1.54Bb              |
| F3                  | 29.48Aa             | 36.84Aab | 30.16Aa | 2.10Cb | 1.42Ca              | 29.75Aa              | 36.74Ab  | 29.73Ba  | 2.29Aa | 1.49Bc              | 29.15Bb              | 37.11Bb | 30.05Aa | 2.15Bb | 1.55Ab              |

A–C Means within a row with different letters are significantly different ( $P < 0.05$ );

a–c Means within a column with different letters are significantly different ( $P < 0.05$ ).

<sup>1</sup> Means of three replications.

<sup>2</sup> Cocoa butter extracted by ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 ml/min of flow rate with unfermented cocoa (F1), partly fermented (F2) and full fermented (F3).

<sup>3</sup> C12:0, C14:0 and C18:3.

Table 6  
Fatty acids composition (area %)<sup>1</sup> changes of cocoa butter extracted by ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 ml/min of flow rate with different roasting treatment and extraction time

| Sample <sup>2</sup> | 5 h Extraction time |          |         |        |                     | 10 h Extraction time |          |         |         |                     | 15 h Extraction time |          |           |         |                     |
|---------------------|---------------------|----------|---------|--------|---------------------|----------------------|----------|---------|---------|---------------------|----------------------|----------|-----------|---------|---------------------|
|                     | C16:0               | C18:0    | C18:1   | C18:2  | Others <sup>3</sup> | C16:0                | C18:0    | C18:1   | C18:2   | Others <sup>3</sup> | C16:0                | C18:0    | C18:1     | C18:2   | Others <sup>3</sup> |
| R1                  | 28.93Ac             | 38.88Ba  | 28.47Ad | 2.29Bc | 1.43Bd              | 28.71Abc             | 39.76Aa  | 27.68Bd | 2.33Acd | 1.52Ac              | 28.97Aab             | 38.24Bc  | 29.28Bcd  | 2.12Cc  | 1.39Cc              |
| R2                  | 29.88Aa             | 37.48Abc | 30.88Aa | 0.22Ce | 1.54Ac              | 28.82Bb              | 37.07Bd  | 30.49Aa | 2.34Abc | 1.27Cf              | 28.66Bb              | 38.86Bbc | 29.03Bd   | 2.08Bd  | 1.37Bc              |
| R3                  | 29.41Ab             | 36.22Ad  | 30.19Ab | 2.29Ac | 1.90Aa              | 28.09Bd              | 38.33Bc  | 29.62Bb | 2.30Ad  | 1.66Ba              | 28.03Bc              | 38.46Ac  | 29.73ABbc | 2.13Bc  | 1.65Ba              |
| R4                  | 28.96Ac             | 37.27Bbc | 30.21Ab | 2.26Bc | 1.30Cf              | 28.59Abc             | 39.07Ab  | 28.51Cc | 2.38Ab  | 1.46Ae              | 28.03Bc              | 39.18Ab  | 29.07Bd   | 2.35Ab  | 1.36Bc              |
| R5                  | 29.01Ac             | 37.08Bbc | 30.15Ab | 2.36Ab | 1.41Be              | 28.04Bd              | 39.90Aa  | 28.20Bc | 2.31Bcd | 1.56Ab              | 27.52Cd              | 40.20Aa  | 28.41Be   | 2.32ABb | 1.56Ab              |
| R6                  | 28.77Ac             | 37.58Bb  | 28.99Bc | 2.79Aa | 1.87Bb              | 28.28Bcd             | 38.00ABc | 30.23Aa | 2.65Ba  | 0.85Ag              | 27.72Bcd             | 38.29Ac  | 30.46Aa   | 2.67Ba  | 0.85Bd              |
| R7                  | 29.48ABb            | 36.84Ac  | 30.16Ab | 2.10Cd | 1.42Cde             | 29.75Aa              | 36.74Ad  | 29.73Ab | 2.29Ad  | 1.49Bd              | 29.15Ba              | 37.11Ad  | 30.05Aab  | 2.15Bc  | 1.55Ab              |

A–C Means within a row with different letters are significantly different ( $P < 0.05$ );

a–f Means within a column with different letters are significantly different ( $P < 0.05$ ).

<sup>1</sup> Means of three replications.

<sup>2</sup> Cocoa butter extracted obtained by ethanol (25% w/w) as cosolvent in SC-CO<sub>2</sub> at 35 MPa, 60 °C and 2 ml/min of flow rate with roasted sample of 120 °C; 15 min (R1), 120 °C; 25 min (R2), 120 °C; 35 min (R3), 150 °C; 15 min (R4), 150 °C; 25 min (R5), 150 °C; 35 min (R6 and unroasted treatment (R7).

<sup>3</sup> C12:0, C14:0 and C18:3.

generally promote more efficient and rapid extraction (Taylor, 1996).

The results of triglycerides and fatty acid methyl esters composition of cocoa butter extracted using supercritical fluid are shown in Tables 1–6. Triglycerides are the major component present in cocoa butter with 92–96% of lipid composition (D'Alonzo, Kozarek, & Wade, 1982; Davis & Dimick, 1989; Lehrian & Keeney, 1980; Lipp & Anklam, 1989). The results showed that cocoa butter extracted from various treatments had three main triglycerides, POS, POP and SOS. POS was the major component with a yield more than 42.52% followed by SOS and POP with more than 28.69% and 18.98% of yield, respectively. As can be seen from the Tables 1–3, the particle size and roasting treatment had significant effect ( $P < 0.05$ ) on the triglycerides composition of cocoa butter, except for POP (S1 and R6), POS (R1), SOS (SR4, R6, R70) and other components (R6). In general, degree of fermentation did not have significant effect ( $P > 0.05$ ) on triglycerides composition, except for POP (F1), SOS (F1) and other components (F1). These findings were in good agreement with the typical triglycerides composition (area %) of cocoa butter obtained by Chaiseri and Dimick (1989).

Results from gas chromatographic analysis showed that palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) were the three main fatty acids in the cocoa butter extracted with stearic acid (33.70–40.22%) being the major component followed palmitic acid (28.03–33.70%) and oleic acid (26.36–30.49%). It can be seen from Tables 4–6 that fatty acids composition of cocoa butter were significantly affected ( $P < 0.05$ ) by the particle size, degree of fermentation and roasting treatment except for all TG (S4), C16:0 (R1), C18:0 (F2, F3 and R7), C18:1 (F1 and R7) and C18:2 (F1). These findings were in good agreement with the typical fatty acids composition (area %) of cocoa butter obtained by Chaiseri and Dimick (1989).

#### 4. Conclusions

Cocoa butter was successfully extracted from cocoa nibs of different particle size, different fermentation level, and different roasting time and temperature using supercritical fluid extraction. The highest yield were obtained from cocoa nibs having smallest particle size (S1 = 0.074 mm) with 92% yield for almost nearly 20 h, unfermented cocoa nibs (F1) with 100% yield for almost nearly 10 h and cocoa nibs roasted at 150 °C and 35 min (R6) with 100% yield for almost nearly 14 h. Increasing roasting time and temperature has resulted in the increase on the yield. Particle size, degree of fermentation and roasting showed significant effect on the yield. Three main triglycerides of cocoa butter were identified in the extract namely glycerol-1-palmitate-2-oleate-3-stearate (POS), glycerol-1,3-dipalmitate-2-oleate (POP) and glycerol-1,3-distearate-2-oleate (SOS), with POS (42.52–46.44%) being the major component. Palmitic, stearic, oleic and linoleic were identified as the main fatty acids present in the extracted cocoa butter, with stearic acid

being the highest component (33.70–40.22%). In general, particle size and roasting showed significant effect on the triglycerides and fatty acids composition, except fermentation. Triglycerides and fatty acids were found to be similar in composition to those of cocoa butter obtained by conventional method.

The supercritical fluid extraction is feasible to be implemented in the cocoa industry. It has been used commercially in the decaffeination of coffee and tea, and the extraction of hops, spices, drugs and natural products. Nevertheless, equipment replacing, from the conventional method of extraction to supercritical fluid may need high cost of investment. The difference in the operational cost may not be significant since the CO<sub>2</sub> and ethanol can be recycled. In addition, higher yield and better quality cocoa butter will be produced.

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